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Supplemental Material

Effects of Periconception Cadmium and Mercury Co-

Administration to Mice on Indices of Chronic Disease in Male

Offspring at Maturity

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Primer	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Product Size (bp)
GLUT4	CTACTCAGGGCTAACATCAGG	CAACCAGAATGCCAATGACGA	118
IRS1	CACCATCTCAACAACCCTCC	GTTTCCCACCCACCATACTG	102
ACACA	GAAGCCAAGATAATCCAGCA	CATACATATCTTTCATCCCACCAG	168
FASN	TGGGTGTGAGTGGTTCAGAG	CAATGCTTGGTCCTTTGAAGTC	140
CD36	TGGCTAAATGAGACTGGGAC	GCAACAAACATCACCACTCC	130
FATP	GAAGTGAATGTGTATGGCGTG	CTCAATGGTATCTTGTATCCTCAG	173
G6PC	GCCTTCTATGTCCTCTTTCCC	AACAGAATCCACTTGAAGACGAG	115

Table S1: List of primers used and listed in forward and reverse direction (5'-3'). Altered mRNA abundance of specific genes was used to assess the impact of periconception Cd and Hg administration on glucose and lipid metabolism in liver and abdominal adipose tissues.

Treatment Control 0.125 mg 0.5 mg2 mg Birth 1.57 ± 0.02 1.63 ± 0.02 1.58 ± 0.03 1.61 ± 0.02 Weight (g) (n=55)(n=51)(n=55)(n=53)18 14 14 14 Litter 1 12 Litter 2 12 15 14 **Litter Size** Litter 3 14 12 13 16 Litter 4 11 10 14 11 Litter 1 18 18 18 19 19 Gestational Litter 2 18 18 18 Length (Days) 19 19 19 Litter 3 19 Litter 4 19 19 18 19

Table S2: Birth weights and litter size of male and female offspring of control and periconception Cd plus Hg-treated female mice and gestation length. There were no significant differences in any of the parameters tested between individual treatment groups and controls. (P>0.05). Data are presented as mean \pm SEM.

Organ Weights at 24 Weeks of Age

Treatment	Liver	Testes	Kidney
Control	2605.41 ± 112.40	271.52 ± 9.33	902.36 ± 37.39
0.125 mg	2723.38 ± 196.19	251.62 ± 6.45	863.47 ± 35.20
0.5 mg	2828.71 ± 110.28	303.91 ± 11.63	939.79 ± 40.38
2 mg	2469.88 ± 90.53	253.46 ± 12.11	958.48 ± 22.73

Table S3: Organ weights of 24-week-old male offspring of control and periconception Cd plus Hg-treated female mice. There were no significant differences in any of the parameters tested between individual treatment groups and controls. (P>0.05; n=16 offspring per treatment). Data are presented as mean \pm SEM.

Treatment	Week12	Week 15	Week 18	Week20	Week22	Week 24
Control	45.55	46.55	45.66	45.51	44.63	44.89
0.125 mg	49.46	50.21	51.59 [*]	52.27^{*}	52.16*	54.86*
0.5 mg	51.19	51.11	51.53*	51.51*	53.66*	57.30 [*]
2 mg	49.43	49.89	50.50*	49.61*	50.17*	51.66*

Table S4: Body weights of male offspring of control and periconception Cd plus Hg-treated female mice in Experiment 1. There were no significant differences in body weights until 15 weeks of age, but body weights were significantly higher in all treatment groups compared to controls after 15 weeks of age until the experiment terminated at 24 weeks of age (* P<0.05 compared to controls; n=16 offspring per treatment).

Treatment	Week13	Week 16	Week 17	Week 22	Week 25
Control	43.09	45.59	46.56	51.16	51.01
2 mg	42.79	46.59	49.25	54.07^{*}	57.44*

Table S5: Body weights of male offspring of control and periconception Cd plus Hg-treated female mice in Experiment 2. Body weights were significantly higher in the treatment group male offspring compared to controls at 22 and 25 weeks of age (* P<0.05 compared to controls; n=19 for controls and n=17 for treated offspring).

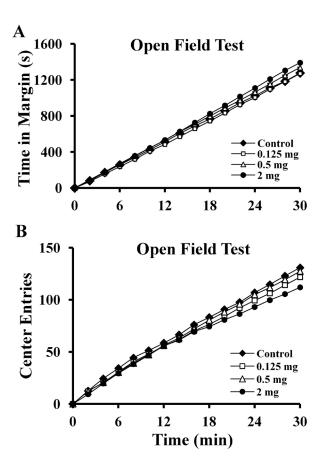


Figure S1: Anxiety-like behavior of eight-week-old male offspring of control and periconception Cd plus Hg-treated female mice. Cumulative amount of time spent in the margin area and entries to the center area were tested by open field tests. Significant differences over time between treatment versus control offspring were tested. (P<0.05 compared to controls; n=8 animals per treatment). (A) Cumulative amount of time spent in the margin area for male offspring. Offspring of 0.5 and 2 mg treatment groups spent significantly more time in the margin area compared to controls (B) Cumulative amount of entries to the center area for male offspring. Offspring of 2 mg treatment group displayed significantly reduced number of entries to the center area compared to controls. X-axis represents total experimental duration of open field test.

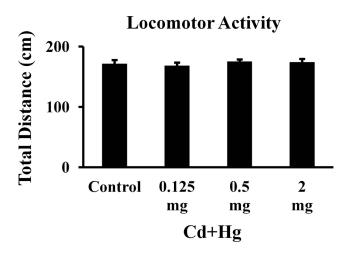


Figure S2: Locomotor activity of male offspring at eight weeks of age. Total movement distance in the open field test system was tested (P>0.05 compared to controls; n=8 per treatment). Data are presented as means \pm SEMs.

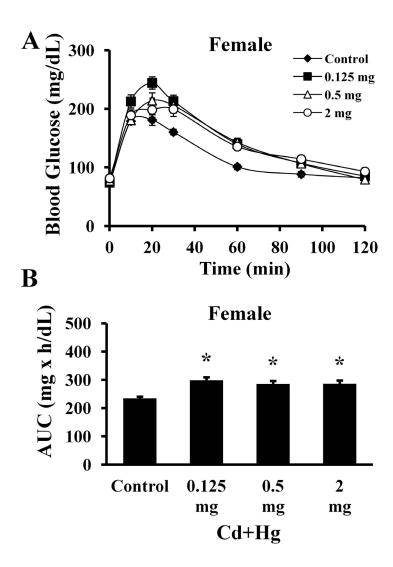
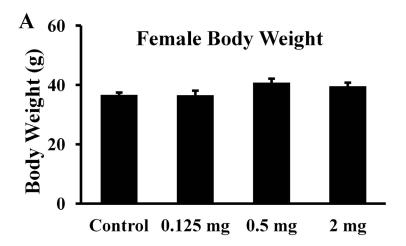


Figure S3: Glucose tolerance test and area under the curve values for 12-week-old female offspring. (A) Glucose tolerance of female offspring (n=14 per treatment). X-axis represents experimental duration in minutes and Y-axis represents blood glucose concentration in mg/dL. Significant differences over time between treatment versus control offspring were tested (P<0.05 compared to controls). (B) Area under the curve values (* P<0.05 compared to controls). X-axis represents each treatment group. Y-axis represents AUC values for plasma glucose in mg x h/dL of blood. Data are presented as mean ± SEM.



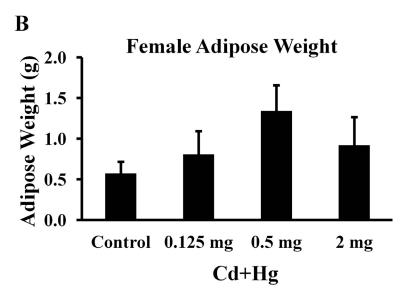


Figure S4: Body and adipose weights of female offspring at 24 weeks of age (P>0.05 compared to controls; n=14 per treatment). (A) Female offspring body weights. (B) Female offspring abdominal adipose weights. X-axis represents each treatment group. Data are presented as mean \pm SEM.